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We want to present some light and electromicrographs from our experimental irradiation material. What we are going to show today has been studied by us (de Estable et al. 1964) with chrome-osmium perfusion fixation utilizing a slight variant of Palay's technique (Palay et al. 1961).

First, we tried to determine the usefulness of this method of fixation to allow parallel light and electronmicroscopical studies, mainly ultrastructural histochemistry of glycogen (de Estable et al. 1963).

Previous investigations have shown the presence of glycogen after ionizing radiation (Klatzo et al. 1961; Miquel et al. 1963; Kruger et al. 1963).

The material that we are going to present correspond to rat brains perfused with chrome-osmium solution after X-ray radiation to the head.

Small pieces of tissue were taken from these brains to be embedded in Epon and the rest was embedded in paraffin for light microscopy. After deparaffination, the sections were treated with dimedone according to Bulmer procedure (1) in order to obtain a selective blocking of no-glycogen aldehyde groups. PAS after this blocking reagent, allows the demonstration of glycogen without the background otherwise present after osmic fixation of nervous tissue; amylase control corroborated the selective action of dimedone.

Our data show the excellency of this procedure for preservation and demonstration of cellular elements in general and particularly of glycogen. Glycogen appears homogenously distributed in the cytoplasm without the coarse appearance, unavoidable artefact after picroalcoholic fixation. Figure 1 shows the periventricular nervous tissue. The blood vessels appear empty and dilated due to the perfusion procedure. Some of the

ependymal cells contain glycogen which appear homogenously distributed in the cytoplasm.

Figure 2 corresponds to the cerebellar cortex of a rat (fixed by osmic perfusion and stained with PAS after dimedone) 24 hours after 20.000 X-ray radiation of the head. The PAS positive material of the molecular layer can be easily attributed to the von bergmann cells being the Purkinje cells spared.

Glycogen is also present in less amount, in some astrocytes of the granular layer, close to the Purkinje cells. In the granular layer pyknotic granule cells are present. This is a well known fact for radiobiologists being the granule cells among the most radiosensible nervous cells.

We don't know yet which is the relationship, if any, between these altered granule cells that have a long process which ramifies in the molecular layer, and the increase of glycogen in the glial cells. Figure 3 shows an area of Figure 2 at a higher magnification. The pyknotic granule cells and the glial glycogen is better illustrated.

Figure 4 is a topographic electronmicrograph that shows the general aspect of the boundary region between myelin core and granular layer of the cerebellar cortex after osmic perfusion. The myelinated fibers, very tightly packed exhibit nitid neurofilaments. At the right bottom, an empty vessel dilated by the perfusion fluid is seen. In the upper part, glial fibers are present.

Figure 5 is an electronmicrograph of the granular layer. Normal granule cells present a big nucleus with nucleoli and scarce cytoplasm with mitrochondria and ribonucleic acid particles, functional equivalent of Nissl granules present in other neurons. Some altered granule cells show a reduction in their diameter due to cytoplasmic retraction and mainly to a nuclear retraction and densification. Clear lacunar areas with granular appearance are present in the nucleus.

At higher magnification in Figure 6, glial processes adjacent to altered granule cells, are loaded with lead stained granules. We interpret

these granules as glycogen due to their size, the positive lead staining and the correlation with the light microscopy observations.

In the molecular layer, the von Bergmann cell processes are loaded with glycogen granules. These granules are also present in astrocytic processes of neuropyl but not in nervous fibers, nor in axonal endings, neither in dendrytic fibers that contact them (Figure 7).

The coexistence of glial fibers and glycogen granules, certifies that glycogen is present in astrocytes.

At present we are doing a time dose study in order to find out the mechanism of glycogen formation as well as its possible relationship to different cell organelles.

LEGENDES

FIGURE 1: Ventricular ependyma of a rat irradiated with a single X-ray dose of 20.000 roentgen, to the head. Fixation by osmic perfusion 24 hours after irradiation. PAS-hematoxillin staining after dimedone pretreatment. The arrows point to the glycogen uniformly distributed in an ependymal cell.

FIGURE 2: Rat cerebellar cortex fixed by osmic perfusion 24 hours after having received a single X-ray dose of 20.000 roentgen. PAS-hematoxilin stain after dimedone. Notice the PAS positive material in the Bergmann cells and their processes.

FIGURE 3: Higher magnification of previous slide. Notice that the glycogen is restricted to glial cells being the Purkinje cells spared. Pyknotic granule cells can be seen.

FIGURE 4: Rat cerebellar cortex fixed by osmic perfusion 24 hours after 20.000rX-radiation of the head. Topographic slide of the boundary region between myelin core and granular layer. Notice the compactness of the tissue without the artefacts present in fixation by immersion. Abundant myelinated fibers are seen as well as glial fibers. Epon embedding. Karnovsky lead stain. X17.000.

FIGURE 5: Rat cerebellar cortex fixed by osmic perfusion 24 hours after 20.000r X-radiation of the head. Granular layer, some granule cells are seen. Arrows point to altered granule cells. Epon embedding. Karnovsky lead stain. X 13.000.

FIGURE 6: Similar area to the previous slide showing a pyknotic granule cell as well as an astrocytic process loaded with glycogen granules. Fixation by osmic perfusion. Epon embedding. Karnovsky lead stain. X.60.000.

FIGURE 7: Rat cerebellar cortex fixed by osmic perfusion 24 hours after 20.000r X-radiation of the head. In the molecular layer a process of a Bergmann cell loaded with glycogen granules . . . . . Epon embedding. Karnovsky lead stain. X.60.000.

FIGURE 8: Similar area than the anterior slide showing the coexistence of glycogen granules and glial fibers. Epon embedding. Karnovsky lead stain. X.80.000.

CODE FOR LEGENDES

G	granule cell
Gl	glycogen
FM	myelinated fiber
EG	glial fiber
CB	Bergmann cell
PA	astrocytic process

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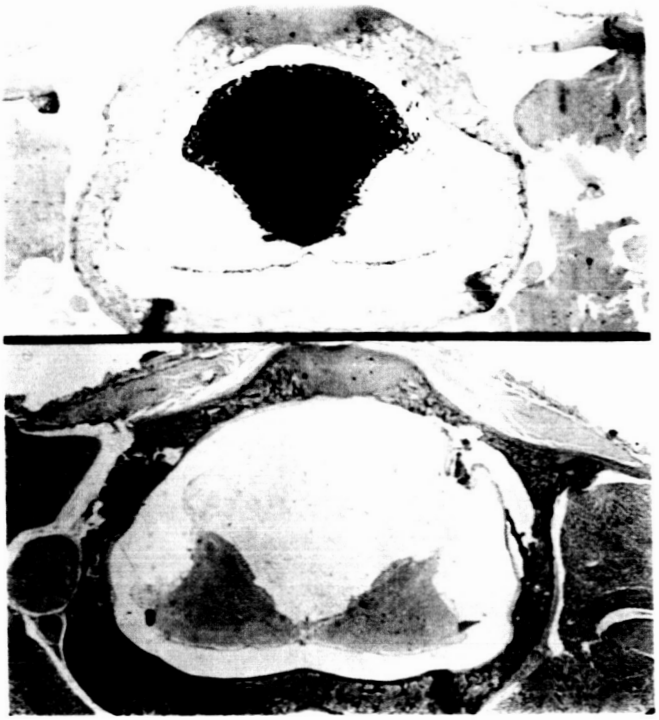
KEY TO ABBREVIATIONS:

G	granule cell	FM	myelinated fiber
Gl	glycogen	FG	glial fiber
CB	von Bergmann cell	PA	Astrocytic process



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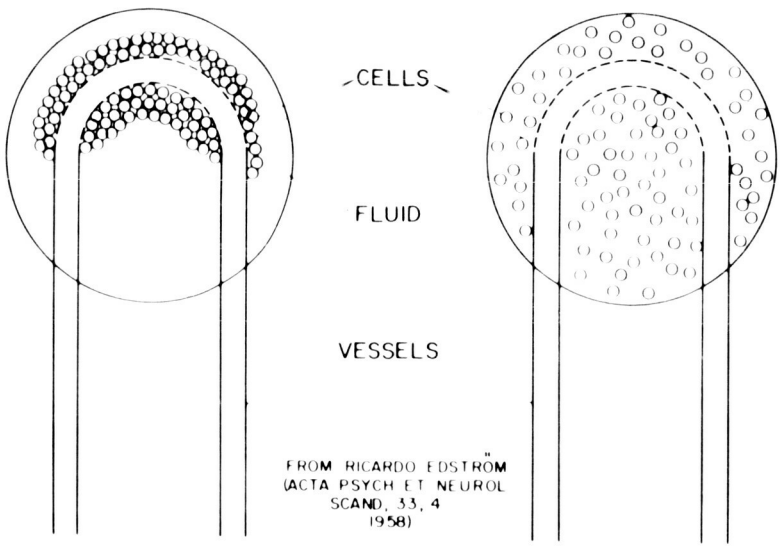
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OTHER ORGAN
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VESSELS
FROM RICARDO EDSTRÖM
(ACTA PSYCH ET NEUROL
SCAND, 33, 4
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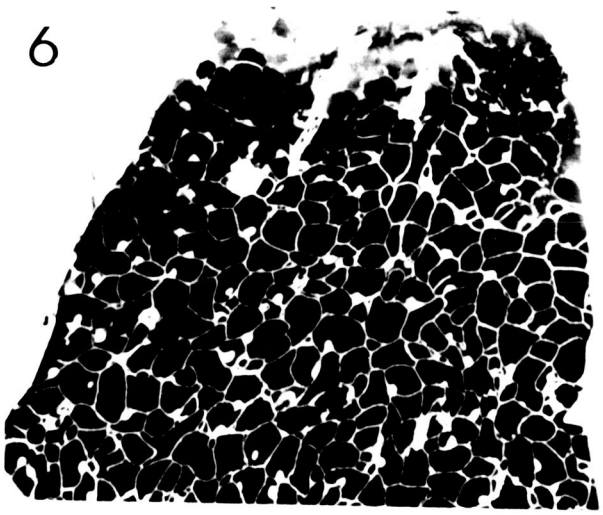

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